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1,2,4-BENZOTRIAZINE OXIDES AS RADIOSENSITIZERS AND SELECTIVE CYTOTOXIC AGENTSPatent Number: ☐ WO8908647Publication
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JP2793312B2, KR140894, NO179005B, NO179005C, NO904003, ☐ PT90039Cited patent(s): WO8802366; US2489359; US2489352; FR2322140; EP0001090; US3482024;
GB1234845; DE2404375

Abstract

A method of using 1,2,4-benzotriazine oxides as radiosensitizers and selective cytotoxic agents is disclosed. The compounds are shown to specifically radiosensitive hypoxic tumor cells and are additionally disclosed to be useful as specific cytotoxic agents for these cells.

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Description

1,2,4-BENZOTRIAZINE OXIDES AS RADIOSENSITIZERS AND SELECTIVE CYTOTOXIC AGENTS

The herein application is a continuation-in-part of U.S. Application Serial No. 911,906, filed 25 September 1986.

Reference to Government Grant or Contract

The invention described herein was made in the course of work under grant or contract from the Department of Health and Human Services. The Government has certain rights in this invention.

Technical Field

The invention relates to cytotoxic agents and radiotherapy effective against hypoxic cells.

Specifically, the invention relates to selectively killing tumor cells and to sensitizing tumor cells to radiation using 1,2,4-benzotriazine oxides.

Background Art

Hypoxic cell radiosensitizers are compounds that selectively increase the sensitivity of hypoxic -ce-ls to destructive radiation. Cytotoxins which have enhanced activity under hypoxic conditions also provide a means for selective destruction of cells under low oxygen pressure. This specificity for hypoxic cells is important because it is tumors that are typically characterized by such cells. Virtually all tumors which are present as solid masses contain these cells, while normal cells generally have an adequate supply of oxygen. Accordingly, anti-tumor agents can be made selective for tumors by virtue of high activity under hypoxic conditions, and radiation can be employed more effectively in the presence of these sensitizers.

Of course, the use of radiation treatment to destroy tumor cells is only practical if damage to the surrounding normal tissue can be minimized or avoided.

The effects of radiation are enhanced by the presence of oxygen, and it is established that as the dose of radiation is increased, the effectiveness of the radiation in destroying target cells is enhanced most dramatically when oxygen is present. Therefore, selectivity for tumor cells toward radiation is difficult to achieve -- normal cells, in view of their oxygen supply, are generally more susceptible to radiation than the target tumor cells. It is therefore desirable to provide a means of sensitizing tumor cells, but not the surrounding tissue, to radiation treatment.

One solution would be to increase the supply of oxygen to these tumor cells. This, however, has proved difficult to do.

Various heterocyclic compounds and in particular those with oxidized nitrogen moieties, have been used to radiosensitize hypoxic tumor cells. Indeed, it has been postulated that the oxidized nitrogen functionality is responsible for this activity.

Nitroimidazoles, particularly misonidazole (MIS) and metronidazole have been studied extensively, and MIS is commonly used as a standard in in vitro and in vivo tests for radiosensitizing activity. (See, e.g., Asquith, et al, Radiation Res (1974) 60:108-118; Hall, et al, Brit J Cancer (1978) 37: 567-569; Brown, et al, Radiation Res (1980) 82:171-190; and U.S. patent 4,371,540. The radiosensitizing activities of certain 1-substituted 3(5)-nitro-1,2,4-triazoles and of various quinoxaline-1,4-dioxide derivatives have also been disclosed.

In addition, US Serial Nos. 730,761, filed 3

May 1985, and 788,762, filed 18 October 1985 assigned to the same assignee and incorporated by reference disclose a group of radiosensitizers that do not contain oxidized nitrogen -- the substituted benzamides and nicotinamides and their thio analogs. These compounds, nevertheless, are radiosensitizers. It is important to distinguish the ability to sensitize hypoxic cells selectively, for instance, by enhancing their oxygen supply, from another mechanism commonly encountered for "sensitizing" cells: inhibition of the enzyme poly(ADP-ribose)polymerase, which is believed to be essential in the repair of irradiated cells after radiation. This repair mechanism is operative in both hypoxic tumor cells and in normal

cells. Hence, administration of "radiosensitizers" which operate according to this latter mechanism does not accomplish the desired purpose of selectively sensitizing the target tumor cells.

A group of compounds which has not previously been suggested for use in either selectively killing hypoxic cells or in radiosensitizing such cells is 3-amino-1,2,4-benzotriazine 1,4-di-N-oxide and related compounds. Related US patents 3,980,779; 3,868,371; and 4,001,410 disclose the preparation of a group of these compounds and their use as anti-microbial agents, particularly by addition of these materials to livestock fodder. US patents 3,991,189 and 3,957,799 disclose derivatives of these compounds bearing substituents on the nitrogen of the 3-amino group. These compounds also have anti-microbial activity.

The present invention provides additional compounds which specifically radiosensitize hypoxic cells in vitro and which, furthermore, are directly cytotoxic to hypoxic cells both in vitro and in vivo.

Therefore, administration of these compounds prior to or following radiation treatment of tumors selectively kills the hypoxic (tumor) cells which survive the radiation dose. Both the ability of these compounds to radiosensitize hypoxic cells in vitro and especially their ability to selectively kill hypoxic cells directly are unexpected properties of these compounds.

Disclosure of the Invention

- The invention provides a valuable addition to the group of compounds currently available as selective radiosensitizers and selective cytotoxic agents for hypoxic tumor cells. Some of the compounds useful in this regard are known compounds, others are novel. One aspect of the invention, therefore, is a method of radiosensitizing or selectively killing hypoxic tumor cells with a compound of the formula:

wherein X is H, hydrocarbyl (1-4C), OH, OR, NH₂, NHR or NR₂ where each R is independently an alkyl of 1-4 carbon atoms, an amide, or a morpholino moiety and may further be substituted with hydroxy, alkoxy, amino, or halogeno substituents;

wherein n is 0 or 1; and

Y₁ and Y₂ are independently either H, halogeno, hydrocarbyl (1-14C) including cyclic and unsaturated hydrocarbyl, optionally substituted with 1 or 2 substituents selected from the group consisting of halogeno, hydroxy, epoxy, alkoxy, alkylthio, amino (including morpholino), acyloxy, acylamido and their thio analogs, alkylsulfonyl, alkylphosphonyl, carboxy, alkoxycarbonyl, carbamyl or alkylcarbamyl, and wherein the hydrocarbyl can optionally be interrupted by a single ether (-O-) linkage, or wherein Y₁ and Y₂ are independently either NHR', O(CO)R', NH(CO)R', O(SO)R', or O(POR)R' in which R' is a hydrocarbyl optionally substituted as defined above.

The compounds of the invention, therefore, are the mono- or dioxides of optionally substituted 1,2,4-benzotriazine which may contain a hydrocarbyl (1-4C), hydroxyl or amino group, either substituted or unsubstituted, in the 3 position. While all of the compounds defined by Formula 1 are generally effective as radiosensitizers, only compounds unsubstituted at the 3-position or having a 3-amino or 3-hydrocarbyl (1-4C) substituent (i.e., XH, hydrocarbyl (1-4C), NH₂, NHR or NR₂ with R as defined above) and which are di-N-oxides (n=1) are effective cytotoxic agents.

Certain of the compounds encompassed by

Formula 1 are already known in the art as being useful for other purposes; other compounds are novel. The novel compounds encompassed by the present invention and which may be prepared by methods disclosed herein include compounds represented by the formula above, in the following three classes: I. X is OH, OR, or NR₂ with R as defined above, n is 0 or 1, and Y₁ and Y₂ are as defined above; II. X is NH₂ or NHR with R as defined above, n is 0, and Y₁ and Y₂ are as defined above; III.

K is NH₂, n is 1 and Y₁ and Y₂ are as defined above but not halogeno, saturated alkyl (1-6C) unsubstituted or halogen-substituted, alkoxy (1-6C), carbamyl, carboxy or carboalkoxy (1-6C); IV. X is H or hydrocarbyl (1-4C), n is 1, and Y₁ and Y₂ are as defined above, with the proviso that when Y₁ and Y₂ are H, X is other than methyl.

Brief Description of the Drawings

Figures 1A, 1B and 1C show the selective cytotoxicity of 3-amino-1,2,4-benzotriazine 1,4-dioxide for hypoxic cells derived from hamster, mouse and human tissues.

Figure 2 shows the in vivo efficacy of 3-amino-1,2,4-benzotriazine 1,4-dioxide in enhancing the killing of tumor cells when combined with radiation.

Figure 3 shows the killing of tumor cells in vivo by 3-amino-1,2,4-benzotriazine 1,4-dioxide when the tumor has been made hypoxic by the intraperitoneal administration of the antihypertensive drug hydralazine.

Modes of Carrying Out the Invention

A. The Compounds Useful in the Invention

The compounds useful in radiosensitizing hypoxic tumor cells as described herein are derivatives of 1,2,4-benzotriazine oxide.

The hydrocarbonyl group represented by Y1 or may contain 1-14 carbon atoms, may be saturated or unsaturated, cyclic or acyclic, and may optionally be interrupted by a single ether linkage. Thus, the unsubstituted form of Y1 or Y2 can be, for example, methyl, ethyl, n-propyl, s-butyl, n-hexyl, 2-methyl-n-pentyl, -2-ethoxyethyl, 3-(n-propoxy)-n-propyl, 4-methoxybutyl, cyclohexyl, tetrahydrofuryl, furfuryl, cyclohexenyl, 3-(n-decyloxy)-n-propyl, 4-methyloctyl, 4,7-dimethyloctyl, and the like.

The hydrocarbonyl may be substituted with one or two substituents as follows: The halogeno substituents are fluoro, chloro, bromo, or iodo. The alkoxy substituents represented by OR' may contain 1 to 4 carbon atoms, and include, for example, methoxy, n-propoxy, and t-butoxy. The amino substituent may be NH₂, NHR or NR₂, where each R is independently an alkyl of 1-4 carbons or a morpholino moiety. R may optionally be substituted with 1-2 hydroxy, alkoxy, amino, or halogeno substituents.

The acyloxy and acylamido groups are represented by R'COO- and R'CONH-, respectively, where R' contains 1-4 carbons, and their thio analogs are represented by R'CSO- and R'CSNH-. Alkyl sulfonyl and alkyl phosphonyl are, respectively, R'SO₂ and R'P(OR')O wherein each R' is independently as above defined.

Carboxy is the group -C(O)OH; alkoxycarbonyl is -C(O)OR'; carbamyl is -C(O)NH₂; and alkylcarbamyl is -C(O)NHR'.

Where X is OH, of course, the compounds may also be prepared and used as the pharmaceutically acceptable salts formed from inorganic bases, such as sodium, potassium, or calcium hydroxide, or from organic bases, such as caffeine, ethylamine, and lysine.

When X is NH₂, pharmaceutically acceptable acid addition salts may be used. These salts are those with inorganic acids such as hydrochloric, hydrobromic or phosphoric acids or organic acids such as acetic acid, pyruvic acid, succinic acid, mandelic acid, p-toluene sulfonic acid, and so forth. (Amino substituents on the hydrocarbonyl side chain can also, of course, be converted to salts.)

The 1,2,4-benzotriazine may be used as the mono- or dioxide. Either the 1-nitrogen of the triazino ring may be oxidized, or both the 1-and 4-nitrogens may be - oxidized.

Specific particularly preferred compounds which are useful in the radiorensitization and cytotoxic procedures of the invention include 3-hydroxy-1,2,4-benzotriazine 1-oxide; 3-hydroxy-1,2,4-benzotriazine 1, 4-dioxide; 3-amino-1,2,4-benzotriazine 1-oxide; 3-amino-1,2,4-benzotriazine 1,4-di-oxide; 6(7)-methoxy-3-hydroxy-1,2,4-benzotriazine 1-oxide; 6(7)-methoxy-3-hydroxy-1,2,4-benzotriazine 1,4-dioxide; 6(7)-methoxy-3-amino-1,2,4-benzotriazine 1-oxide; 6(7)-methoxy-3-amino-1,2,4-benzotriazine 1,4-dioxide; 6(7)-ethoxy-3-hydroxy-1,2,4-benzotriazine 1-oxide; 6(7)-ethoxy-3-hydroxy-1,2,4-benzotriazine 1,4-dioxide; 6(7)-ethoxy-3-amino-1,2,4-benzotriazine 1-oxide; 6(7)-ethoxy-3-amino-1,2,4-benzotriazine 1,4-dioxide; 6(7)-[4-acetamido-n-butoxy]-3-hydroxy-1,2,4 benzotriazine oxide; 6(7)-[4-acetamido-n-butoxy]-3-hydroxy-1,2,4 benzotriazine 1,4-dioxide; 6(7)-[4-acetamido-n-butoxy]-3-amino-1,2,4 benzotriazine 1-oxide; 6(7)-[4-acetamido-n-butoxy]-3-amino-1,2,4 benzotriazine 1,4-dioxide; 6(7)-[1-(2,3-dihydroxy)propoxy]-3-hydroxy-1,2,4 benzotriazine 1-oxide; 6(7)-[1-(2,3-dihydroxy)propoxy]-3-hydroxy-1,2,4 benzotriazine 1,4-dioxide; 6(7)-[1-(2,3-dihydroxy)propoxy]-3-amino-1,2,4 benzotriazine oxide; 6(7)-[1-(2,3-dihydroxy)propoxy]-3-amino-1,2,4 benzotriazine 1,4-dioxide; 6(7)-[(2-furyl)methylamino]-3-hydroxy-1,2,4 benzotriazine 1-oxide; 6(7)-[(2-furyl)methylamino]-3-hydroxy-1,2,4 benzotriazine 1,4-dioxide; 6(7)-[(2-furyl)methylamino]-3-amino-1,2,4 benzotriazine 1,4-dioxide; 6(7)-[(2-furyl)methylamino]-3-amino-1,2,4 benzotriazine 1,4-dioxide.

benzotriazine oxide, 6(7)-[(2-furyl)methylamino]-3-amino-1,2,4-benzotriazine
 1,4-dioxide; 6(7)-(2-methoxyethylamino)-3-hydroxy-1,2,4-
 benzotriazine oxide; 6(7)-(2-methoxyethylamino)-3-hydroxy-1,2,4-benzotriazine
 1,4-dioxide; 6(7)-(2-methoxyethylamino)-3-amino-1,2,4-benzotriazine
 l-oxide; 6(7)-(2-methoxyethylamino)-3-amino-1,2,4-benzotriazine
 1,4-dioxide; 6(7)-carbethoxymethoxy-3-hydroxy-1,2,4-benzotriazine
 oxide; 6(7)-carbethoxymethoxy-3-hydroxy-1,2,4-benzotriazine
 1,4-dioxide; 6(7)-carbethoxymethoxy-3-amino-1,2,4-benzotriazine
 l-oxide; 6(7)-carbethoxymethoxy-3-amino-1,2,4-benzotriazine
 1,4-dioxide; 6(7)-[(2-methoxyethyl)carbonylmethoxy]-3-hydroxy-1,2,4-
 benzotriazine oxide; 6(7)-[(2-methoxyethyl)carbonylmethoxy]-3-hydroxy-1,2,4-
 benzotriazine 1,4-dioxide; 6(7)-E(2-methoxyethyl)carbonylmethoxy]-3-amino-1,2,4-
 benzotriazine oxide; 6(7)-t(2-methoxyethyl)carbonylmethoxy]-3-amino-1,2,4-
 benzotriazine 1,4-dioxide; 6(7)-[(2-hydroxyethyl)carbonylmethoxy]-3-hydroxy-1,2,4-
 benzotriazine l-oxide; 6(7)-r(2-hydroxyethyl)carbonylmethoxy]-3-hydroxy-1,2,4-
 benzotriazine 1,4-dioxide; 6(7)-[(2-hydroxyethyl)carbonylmethoxy]-3-amino-1,2,4-
 benzotriazine oxide; 6(7)-[(2-hydroxyethyl)carbonylmethoxy]-3-amino-1,2,4-
 benzotriazine 1,4-dioxide; 6(7)-[1-(2-hydroxy-3-morpholino)propoxy]-3-hydroxy-1,2,4-benzotriazine oxide; 6
 (7)-[1-(2-hydroxy-3-morpholino)propoxy]-3-hydroxy-1,2,4-benzotriazine 1,4-dioxide; 6(7)-[1-(2-hydroxy-3-morpholino)propoxy]-3-amino-1,2,4-
 benzotriazine oxide; 6(7)-Cl(2-hydroxy-3-morpholino)propoxy]-3-amino-1,2,4-
 benzotriazine 1,4-dioxide; 6(7)-[3-amino-n-propoxy]-3-hydroxy-1,2,4-benzotriazine l-oxide; 6(7)-[3-amino-n-
 propoxy]-3-hydroxy-1,2,4-benzotriazine 1,4-dioxide; 6(7)-E3-amino-n-propoxy]-3-amino-1,2,4-
 benzotriazine
 oxide; 6(7)-C3-amino-n-propoxy]-3-amino-1,2,4-benzotriazine
 1,4-dioxide; 6(7)-[2,3-epoxypropoxy]-3-hydroxy-1,2,4-benzotriazine
 l-oxide; 6(7)-C2,3-epoxypropoxy]-3-hydroxy-1,2,4-benzotriazine
 1,4-dioxide; 6(7)-[2,3-epoxypropoxy]-3-amino-1,2,4-benzotriazine
 l-oxide; 6(7)-[2,3-epoxypropoxy]-3-amino-1,2,4-benzotriazine 1,4-dioxide; 6(7)-[3-methoxy-2-hydroxy-n-
 propoxy]-3-hydroxy-1,2,4-
 benzotriazine l-oxide; 6(7)-[3-methoxy-2-hydroxy-n-propoxy]-3-hydroxy-1,2,4-
 benzotriazine 1,4-dioxide; 6(7)-[3-methoxy-2-hydroxy-n-propoxy]-3-amino-1,2,4-
 benzotriazine l-oxide; 6(7)-[3-methoxy-2-hydroxy-n-propoxy]-3-amino-1,2,4-
 benzotriazine 1,4-dioxide; 6(7)-[4-ethoxy-3-hydroxy-n-butoxy]-3-hydroxy-1,2,4-
 benzotriazine l-oxide; 6(7)-[4-ethoxy-3-hydroxy-n-butoxy]-3-hydroxy-1,2,4-
 benzotriazine 1,4-dioxide; 6(7)-[4-ethoxy-3-hydroxy-n-butoxy]-3-amino-1,2,4-
 benzotriazine l-oxide; 6(7)-[4-ethoxy-3-hydroxy-n-butoxy]-3-amino-1,2,4-
 benzotriazine 1,4-dioxide; 6(7)-[3,4-dihydroxy-n-butoxy]-3-hydroxy-1,2,4-
 benzotriazine l-oxide; 6(7)-[3,4-dihydroxy-n-butoxy]-3-hydroxy-1,2,4-
 benzotriazine 1,4-dioxide; 6(7)-[3,4-dihydroxy-n-butoxy]-3-amino-1,2,4-
 benzotriazine l-oxide; 6(7)-[3,4-dihydroxy-n-butoxy]-3-amino-1,2,4-
 benzotriazine 1,4-dioxide; 6(7)-methyl-3-hydroxy-1,2,4-benzotriazine 1-oxide; 6(7)-methyl-3-hydroxy-1,2,4-
 benzotriazine 1,4-dioxide; 6(7)-methyl-3-amino-1,2,4-benzotriazine 1-oxide; 6(7)-methyl-3-amino-1,2,4-
 benzotriazine 1,4-dioxide; 6(7)-ethyl-3-hydroxy-1,2,4-benzotriazine 1-oxide; 6(7)-ethyl-3-hydroxy-1,2,4-
 benzotriazine 1,4-dioxide; 6(7)-ethyl-3-amino-1,2,4-benzotriazine 1-oxide; 6(7)-ethyl-3-amino-1,2,4-
 benzotriazine 1,4-dioxide; 6(7)-chloroacetamido-3-hydroxy-1,2,4-benzotriazine
 1-oxide; 6(7)-chloroacetamido-3-hydroxy-1,2,4-benzotriazine
 1,4-dioxide; 6(7)-chloroacetamido-3-amino-1,2,4-benzotriazine
 oxide; 6(7)-chloroacetamido-3-amino-1,2,4-benzotriazine
 1,4-dioxide; 6(7)-[(2-hydroxyethyloxy)acetamido]-3-hydroxy-1,2,4-
 benzotriazine 1-oxide; 6(7)-t(2-hydroxyethyloxy)acetamido]-3-hydroxy-1,2,4-
 benzotriazine 1,4-dioxide; 6(7)-t(2-hydroxyethyloxy)acetamido]-3-amino-1,2,4-
 benzotriazine oxide; 6(7)-[(2-hydroxyethyloxy)acetamido]-3-amino-1,2,4-
 benzotriazine 1,4-dioxide; 6,7-dimethoxy-3-hydroxy-1,2,4-benzotriazine 1-oxide; 6,7-dimethoxy-3-hydroxy-
 1,2,4-benzotriazine 1,4-dioxide; 6,7-dimethoxy-3-amino-1,2,4-benzotriazine 1-oxide; 6,7-dimethoxy-3-
 amino-1,2,4-benzotriazine 1,4-dioxide; 6,7-diethoxy-3-hydroxy-1,2,4-benzotriazine 1-oxide; 6,7-diethoxy-3-
 hydroxy-1,2,4-benzotriazine 1,4-dioxide; 6,7-diethoxy-3-amino-1,2,4-benzotriazine 1-oxide; 6,7-diethoxy-3-
 amino-1,2,4-benzotriazine 1,4-dioxide; 6(7)-propionyl-3-hydroxy-1,2,4-benzotriazine l-oxide; 6(7)-propionyl-
 3-hydroxy-1,2,4-benzotriazine 1,4-dioxide; 6(7)-propionyl-3-amino-1,2,4-benzotriazine 1-oxide; 6(7)-
 propionyl-3-amino-1,2,4-benzotriazine 1,4-dioxide; 6(7)-(2-acetoxyethoxy)-3-hydroxy-1,2,4-benzotriazine

l-oxide; 6(7)-(2-acetoxyethoxy)-3-hydroxy-1,2,4-benzotriazine
 1,4-dioxide, 6(9)-(2-acetoxyethoxy)-3-amino-1,2,4-benzotriazine
 l-oxide; 6(9)-(2-acetoxyethoxy)-3-amino-1,2,4-benzotriazine
 1,4-dioxide; 6(7)-n-hexyloxy-3-hydroxy-1,2,4-benzotriazine l-oxide; 6(7)-n-hexyloxy-3-hydroxy-1,2,4-benzotriazine
 1,4-dioxide; 6(7)-n-hexyloxy-3-amino-1,2,4-benzotriazine 1-oxide; 6(7)-n-hexyloxy-3-amino-1,2,4-benzotriazine 1,4-dioxide; 6(7)-ethylamino-3-hydroxy-1,2,4-benzotriazine oxide, 6(7)-ethylamino-3-hydroxy-1,2,4-benzotriazine
 1,4-dioxide; 6(7)-ethylamino-3-amino-1,2,4-benzotriazine 1-oxide; 6(7)-ethylamino-3-amino-1,2,4-benzotriazine 1,4-dioxide; 6(7)-(2-methoxyethoxy)-3-hydroxy-1,2,4-benzotriazine
 1-oxide; 6(7)-(2-methoxyethoxy)-3-hydroxy-1,2,4-benzotriazine
 1,4-dioxide; 6(7)-(2-methoxyethoxy)-3-amino-1,2,4-benzotriazine
 l-oxide; 6(7)-(2-methoxyethoxy)-3-amino-1,2,4-benzotriazine
 1,4-dioxide; 6(7)-(aminoacetamido)-3-hydroxy-1,2,4-benzotriazine
 l-oxide; 6(7)-(aminoacetamido)-3-hydroxy-1,2,4-benzotriazine
 1,4-dioxide; 6(7)-(aminoacetamido)-3-amino-1,2,4-benzotriazine
 l-oxide; 6(7)-(aminoacetamido)-3-amino-1,2,4-benzotriazine
 1,4-dioxide; 6(7)-(carbamylmethoxy)-3-hydroxy-1,2,4-benzotriazine oxide; 6(7)-(carbamylmethoxy)-3-hydroxy-1,2,4-benzotriazine
 - 1,4-dioxide; 6(7)-(carbamylmethoxy)-3-amino-1,2,4-benzotriazine
 1-oxide; 6(7)-(carbamylmethoxy)-3-amino-1,2,4-benzotriazine
 1,4-dioxide; 6(7)-(carboxymethoxy)-3-hydroxy-1,2,4-benzotriazine
 oxide, 6(7)-(carboxymethoxy)-3-hydroxy-1,2,4-benzotriazine
 - 1,4-dioxide; 6(7)-(carboxymethoxy)-3-amino-1,2,4-benzotriazine oxide; 6(7)-(carboxymethoxy)-3-amino-1,2,4-benzotriazine
 1,4-dioxide; 6(7)-[1,2-dihydroxyethyl]-3-amino-1,2,4-benzotriazine
 1,4-dioxide; 6(7)-[1-(3-ethylamino-2-hydroxypropoxy)]-3-amino-1,2,4-benzotriazine 1,4-dioxide; 6(7)-[2-ethylamino-1-hydroxyethyl]-3-amino-1,2,4-benzotriazine 1,4-dioxide; 6(7)-[2-hydroxyethyl]-3-amino-1,2,4-benzotriazine
 1,4-dioxide; 6(7)-[1-hydroxyethyl]-3-amino-1,2,4-benzotriazine
 1,4-dioxide; 3-(2-hydroxyethylamino)-1,2,4-benzotriazine l-oxide; 3-(2-hydroxyethylamino)-1,2,4-benzotriazine
 1,4-dioxide; 6(7)-chloro-3-(2-hydroxyethylamino)-1,2,4-benzotriazine
 l-oxide; 6(7)-chloro-3-(2-hydroxyethylamino)-1,2,4-benzotriazine
 1,4-dioxide; 3-(1-hydroxyethylamino)-1,2,4-benzotriazine 1-oxide; 3-(1-hydroxyethylamino)-1,2,4-benzotriazine
 1,4-dioxide; 1,2,4-benzotriazine oxide; 1,2,4-benzotriazine 1,4-dioxide; 3-methyl-1,2,4-benzotriazine 1,4-dioxide; 3-ethyl-1,2,4-benzotriazine 1,4-dioxide; 3-propyl-1,2,4-benzotriazine 1,4-dioxide; 6(7)-amino-1,2,4-benzotriazine 1,4-dioxide; 6(7)-amino-3-methyl-1,2,4-benzotriazine 1,4-dioxide; 6(7)-amino-3-ethyl-1,2,4-benzotriazine 1,4-dioxide; 6(7)-methoxy-1,2,4-benzotriazine 1,4-dioxide; 6(7)-methoxy-3-methyl-1,2,4-benzotriazine 1,4-dioxide; 6(7)-[1-(2,3-dihydroxypropoxy)]-1,2,4-benzotriazine
 1,4-dioxide; 6(7)-[1,2-dihydroxyethyl]-1,2,4-benzotriazine 1,4-dioxide; 6(7)-[1-(3-ethylamino-2-hydroxypropoxy)]-1,2,4-benzotriazine 1,4-dioxide; 6(7)-[2-ethylamino-1-hydroxyethyl]-1,2,4-benzotriazine
 1,4-dioxide; 6(7)-chloro-1,2,4-benzotriazine 1,4-dioxide; 6(7)-[2-hydroxyethyl]-1,2,4-benzotriazine 1,4-dioxide; 6(7)-[1-hydroxyethyl]-1,2,4-benzotriazine 1,4-dioxide; and their pharmaceutically acceptable salts and the thioamide analogs of the foregoing list of compounds. It should be noted that the "yl or Y2" substituents set forth in most of the above compounds as present in either the 6 or 7 positions (designated "6(7)") or in both the 6 and 7 positions (designated "6,7") may also be present at the 5 and/or 8 ring positions.

Of the above compounds useful in the method of the present invention as selective cytotoxic agents or radiosensitizers, the following compounds are novel: compounds given by the formula above wherein I. X is

OH, OR, or NR₂, where each R is independently an alkyl of 1-4 carbon atoms, an amide, or a morpholino moiety and may further be substituted with hydroxy, alkoxy, amino, or halogeno substituents, n is 0 or 1, and yl and Y2 are independently either H, halogeno, hydrocarbyl (1-14C) including cyclic and unsaturated hydrocarbyl, optionally substituted with 1 or 2 substituents selected from the group consisting of halogeno, hydroxy, epoxy, alkoxy, alkylthio, amino (including morpholino), acyloxy, acylamido and their thio analogs, alkylsulfonyl, alkylphosphonyl, carboxy, alkoxycarbonyl, carbamyl or alkylcarbamyl, and wherein the

hydrocarbyl can optionally be interrupted by a single ether (-O-) linkage, or wherein yl and Y are independently either NHR' , $\text{O}(\text{CO})\text{R}'$, $\text{NH}(\text{CO})\text{R}'$, $\text{O}(\text{SO})\text{R}'$, or $\text{O}(\text{POR})\text{R}'$ in which R' is a hydrocarbyl optionally substituted as defined above; II.X is NH_2 or NHR with R as defined above, n is 0, and yl and Yz are as defined in I; III. X is NH_2 , n is 1, and Y and Y2 are independently either H, hydrocarbyl (7-14C; saturated or unsaturated), unsaturated hydrocarbyl (1-6C), either hydrocarbyl substituent being either unsubstituted or substituted with halogen, hydroxy, epoxy, alkoxy, alkylthio, amino (including morpholino), acyloxy, acylamido and their thio analogs, alkylsulfonyl or alkylphosphonyl, and wherein the hydrocarbyl can optionally be interrupted by a single ether (-O-) linkage, or wherein yl and Y2 are independently either NHR' , $\text{O}(\text{CO})\text{R}'$, $\text{NH}(\text{CO})\text{R}'$, $\text{O}(\text{SO})\text{R}'$, or $\text{O}(\text{POR})\text{R}'$ in which R' is a hydrocarbyl optionally substituted as defined above; IV.X is H or hydrocarbyl (1-4C), n is 1, and yl and y2 are as defined above, with the proviso that when yl and y2 are H, X is other than methyl.

B. Preparation of the Compounds of the Invention

General methods for preparing some 3-amino derivatives are found in the above reference patents to Ley et al., for example US 3,980,779. The compounds are prepared from benzofuroxan of the formula:

by reaction with a salt of cyanamide, followed by acidification of the reaction mixture. The benzofuroxan starting material is not symmetric with respect to its own 5 and 6 positions (which are the 6 and 7 positions of the resulting 3-amino benzotriazine oxide).

Therefore, a mixture of the 6- and 7-substituted materials may result. If desired, this mixture can be separated using conventional means into individual components having a substituent in either the 6 or 7 position.

The dioxide may also be prepared from the parent monoxide or 1,2,4-benzotriazine by peracid oxidation (see Robbins et al, J Chem Soc 3186 (1957) and Mason et al, J Chem Soc B 911 (1970)).

In addition, the monoxide may be prepared by:

- (1) cyclization of a 1-nitro-2-aminobenzene compound using H_2NCN ;
- (2) oxidation of the parent compound given by the structure

or by controlled reduction of the corresponding dioxide (see Mason, supra, and Wolf et al, J Am Chem Soc 76:355 (1954)).

The 1,2,4-benzotriazines may be prepared by cyclization of formazan precursors using BF_3/AcOH (see Scheme I and Atallah and NazerF Tetrahedron 38:1793 (-1982)). -

3-amino-1,2,4-benzotriazines may be prepared either by cyclization of a parent compound (see Scheme II and Arndt, Chem. Ber. 3522 (1913)) or by reduction of the monoxide or dioxide as above.

The 3-hydroxy-1,2,4-benzotriazine oxides may be prepared using peroxide and tungsten oxide (Scheme III), a novel synthetic procedure for making the 3-hydroxy-1,4-dioxide compound, or concentrated sulfuric acid and sodium nitrate (Scheme IV).

Scheme I

Scheme It

Scheme III

Scheme IV

C. Formulation and Administration

As demonstrated below, the oxidized benzotriazines of the invention may be used to radiosensitize or selectively kill hypoxic tumor cells in warm-blooded animal hosts. A way in which they may be used is in conjunction with agents known to selectively create hypoxia in tumors. Such methods include the use of

antihypertensive drugs such as hydralazine, or agents which affect the amount of oxygen carried by the blood. While these compounds will typically be used in cancer therapy of human patients, they may be used to kill hypoxic tumor cells in other warm blooded animal species such as other primates, farm animals such as cattle, and sports animals and pets such as horses, dogs, and cats.

Hypoxia is believed to be associated with all types of solid malignant neoplasms. The compounds of the invention may, therefore, be used to radiosensitize or to kill neoplastic epithelial cells, endothelial cells, connective tissue cells, bone cells, muscle cells, nerve cells, and brain cells. Examples of carcinomas and sarcomas include carcinomas such as epithelial cell, acidic cell, alveolar cell, basal cell, basal squamous cell, cervical, renal, liver, Hurthle,

Lucke, mucinous and Walker, and sarcomas such as

Abernathy's, alveolar soft part, angiolithic, botryoid, encephaloid, endometria stroma, Ewing's fascicular, giant cell, lymphatic, Jensen's, juxtacortical osteogenic, Kaposi's, medullary, and synovial. Specific examples of tumors that have been sensitized with other radiosensitizers are reported in Adams, G.E.,

Cancer: A

Comprehensive Treatise (F. Becker, Ed) vol 6, pp 181-223, Plenum, New York, 1977.

The compounds may be administered to patients orally or parenterally (intravenously, subcutaneously, intramuscularly, intraspinally, intraperitoneally, and the like). When administered parenterally the compounds will normally be formulated in a unit dosage injectable form (solution, suspension, emulsion) with a pharmaceutically acceptable vehicle. Such vehicles are typically nontoxic and nontherapeutic. Examples of such vehicles are water, aqueous vehicles such as saline, Ringer's solution, dextrose solution, and Hank's solution and nonaqueous vehicles such as fixed oils (e.g., corn, cottonseed, peanut, and sesame), ethyl oleate, and isopropyl myristate. Sterile saline is a preferred vehicle and the compounds are sufficiently water soluble to provide a solution for all foreseeable needs. The vehicle may contain minor amounts of additives such as substances that enhance solubility, isotonicity, and chemical stability, e.g., antioxidants, buffers, and preservatives. When administered orally (or rectally) the compounds will usually be formulated into a unit dosage form such as a tablet, capsule, suppository or cachet. Such formulations typically include a solid, semisolid or liquid carrier or diluent.

Exemplary diluents and vehicles are lactose, dextrose, sucrose, sorbitol, mannitol, starches, gum acacia, calcium phosphate, mineral oil, cocoa butter, oil of theobroma, aginates, tragacanth, gelatin, syrup, methylcellulose, polyoxyethylene sorbitan monolaurate, methyl hydroxybenzoate, propyl hydroxybenzoate, talc, and magnesium stearate.

The amount of compound administered to the subject is sufficient to radiosensitize or to produce cytotoxicity in the malignant neoplasm to be treated but below that which may elicit toxic effects. This amount will depend upon the type of tumor, the species of the subject being treated, the indication dosage intended and the weight or body surface of the subject. The radiation may be administered to humans in a variety of different fractionation regimes, i.e., the total radiation dose is given in portions over a period of several days to several weeks. These are most likely to vary from daily (i.e., five times per week) doses for up to six weeks, to once weekly doses for four to six weeks. An individual dose of the benzotriazine will be given before or after each radiation treatment and is likely to be in the range of 0.01 to 20 mmol/kg and usually in the range of 0.1 to 2 mmol/kg.

For use as selective cytotoxic agents, the compounds of the invention can be administered alone, with radiation or other cancer cytotoxic agents, with vasoactive drugs (e.g., hydralazine), or with procedures which reduce the amount of available oxygen carried by the blood such as anemia or drugs which increase the binding of oxygen to hemoglobin, all of which can enhance selectively the degree of hypoxia in the tumor.

As noted above, while all of the compound encompassed by Formula 1 are generally useful as radiosensitizers herein, only those compounds which are 3-substituted-1,2,4-benzotriazine 1,4-dioxides (i.e.,

X=H, hydrocarbyl (1-4C), NH₂, NHR or NR₂ with R as defined above and n is 1) are useful as selective cytotoxic agents.

Examples

The following examples further illustrate the compounds of the invention and methods for synthesizing and using them, and are not intended to limit the invention in any manner.

Example 1: Preparation of 3-Hydroxy-1,2,4-Benzotriazine 1,4 Dioxide

A stirred mixture of 1.50g (9.25 mmole) of 3-amino-1,2,4-benzotriazine 1-oxide (1), 100.0 ml acetic acid, and 30.0 ml of 30% hydrogen peroxide was treated with 3.05 g (9.25 mmole) of Na₂N₂O₄ 2H₂O. The mixture was stirred in an oil bath at 60°C for 4 days. The yellowish orange mixture was cooled to about 30°C and filtered to remove a light yellow non-UV absorbing solid that was presumably tungstic acid. The orange solution of hydrogen peroxide in acetic acid was evaporated to semi-dryness carefully with several additions of water and acetic acid to remove most of the peroxide. The concentrated solution was allowed to stand at room temperature to afford four crops of an orange solid, 0.87g (42% yield of the sodium salt of 2). UVmax (20% CH₃OH/H₂O): 262.2 (# 39,460); 477 (# 7,030). IR (neat): 3530, 3150, 2650, 2180 and 1635. Anal.

(calculated for the sodium salt): C₇H₄N₃O₃Na 1.25H₂O, 223.64: C, 37.6; H, 2.93; N, 18.79. Found: C, 37.8; H, 2.75; N, 18.65.

Example 2: Preparation of 3-Amino-7-Trifluoromethyl- 1,2,4-Benzotriazine 1-Oxide:

A solution of Na (1.13g, 49.2 mmole) in ethanol (50 ml) was added to a solution of guanidine hydrochloride (4.93g, 51.6 mmole) in ethanol (50 ml).

After 1h, the mixture was filtered and the filtrate was combined with a solution of 4-chloro-3-nitro-benzotrifluoride (Aldrich, 5.5g, 24.4 mmole) in ethanol (25 ml). The mixture was stirred and refluxed for 5 h, cooled to 0-5°C, and the precipitated solid collected.

The solid was washed with water and ethanol and air-dried to give 0.48g (9%) of 3 as a light yellow solid, mp 300°C. TLC: R_f 0.60 (9:1 methylene chloride: methanol on silica gel plates). Mass. Spec.: M⁺=230 (q = 100).

Example 3: Preparation of 3-Amino-7-Decyl-1,2,4-Benzotriazine 1-Oxide

Preparation of 4-(1-decyl)-2-nitroaniline:

Acetic anhydride (400 ml) was added over a 30-minute period to a stirred solution of 4-decylaniline (Aldrich, 80g, 0.34 mole) in hexanes (2.4l). After stirring for 1h, the mixture was cooled and treated over 30 min. at 5-10°C with 70% nitric acid (34 ml). Stirring was continued at 5-10°C for 1h and at 25°C for 16h. The mixture was diluted with H₂O (1l), stirred for 5h, poured into an open dish and allowed to stand for 16h.

After further dilution with H₂O (1.5l), the solid was collected and recrystallized from an 85% ethanol solution (in water) to give 92g (84%) of the intermediate as an orange solid, m.p. 64°C.

A solution (100 ml) of 85% KOH (19g, 0.288 mole) in H₂O was combined with a suspension of 4-(1-decyl)-2-nitroaniline (89g, 0.28 mole), prepared above, in methanol (900 ml). - The mixture was stirred for 6h, neutralized to pH 7-8 with concentrated HCl, and evaporated in vacuo to near dryness. After dilution with H₂O (400 ml), the solid was collected and air-dried to give 77g (100%) of the intermediate as an orange solid, mp 59°C.

1.09 (8.7 mmole) of chloroamidinium hydrochloride (previously prepared for use by treating an ether solution of cyanamide with HCl gas and collecting the precipitated solid) was added portionwise over 10 min to a preheated melt (190°C) of 4-(1-decyl)-2-nitroaniline prepared in the preceding step (500 mg, 1-8 mmole). The reaction mixture was heated at 190°C for 5 min, cooled to 25°C, treated with 6N KOH (10 ml), and heated at 90-95°C for 1h. After cooling to 25°C, the solid was collected, washed with H₂O and ethanol and air-dried to give 0.25g (46%) of compound 4 as a light yellow solid, m.p. 177°C (dec).

Mass. spec. M⁺=285 (q=100), 302 (q=13).

Example 4: Preparation of 3-Amino-7-Carbamyl-1,2,4-Benzotriazine 1-Oxide

Preparation of 4-chloro-3-nitrobenzamide: 20.2g (0.1 mole) of 4-chloro-3-nitrobenzoic acid (Aldrich) and thionyl chloride (20 ml) were combined, allowed to stand for 16h, and refluxed for 4h to give a clear red solution. The solution was evaporated in vacuo and azeotroped with benzene. The residue was dissolved in acetonitrile (20 ml) and added over 30 min to cold (-100C) concentrated ammonium hydroxide (100 ml). After 3h at -100C and 16h at 250C the mixture was poured into an open dish and allowed to evaporate to dryness. The residue was slurried in H₂O and the solid was collected and air-dried to give 19.8g (98%) of the intermediate as a light yellow solid, m.p. 1530C.

A solution of Na (3.45g, 0.15 mole) in ethanol (75 ml) was added to a solution of guanidine hydrochloride (15.8g, 0.165 mole) in ethanol (75 ml).

After 1h the mixture was filtered and the filtrate was combined with a suspension of 4-chloro-3-nitrobenzamide (10g, 0.05 mole) prepared above, in ethanol (50 ml). The mixture was stirred and refluxed for 16h, cooled to 0-50C, and acidified with concentrated HCl (8 ml). The collected solid was combined with K₂CO₃ (28g, 0.2 mole) and H₂O (40 ml) and the mixture was stirred and heated at 1000C for 8h. After cooling to 250C, the solid was collected, washed with H₂O, and air-dried. The solid was suspended in boiling ethyl acetate, collected and washed with hot ethyl acetate. The solid was repeatedly suspended in boiling dioxane and collected (6x100ml).

The combined filtrate was evaporated in vacuo to a solid. The solid was suspended in 95% ethanol, collected and air-dried to give 0.44g (4.3%) of compound 5 as a light yellow solid, m.p. 300aC. TLC: R_f=0.23 (methylene chloride: acetone of 2:1, silica gel plates).

Mass. Spec.: M+ 205 (q= 100).

Example 5: Preparation of 7-Acetyl-3-Amino-1,2,4-Benzotriazine 1-Oxide Oxime

A combined mixture of 7-acetyl-3-amino-1,2,4-benzotriazine oxide (prepared in Example 5; 50 mg, 0.25 mmole), hydroxylamine hydrochloride (200 mg, 2.88 mmole), pyridine (1 ml), and ethanol (1 ml) was heated at 90-950C for 1h and then cooled to 250C. The mixture was diluted with 95% ethanol (5 ml) and the solid was collected and air-dried to give 30 mg (56%) of compound 6 as a light yellow solid, m.p. 2780C (dec).

TLC: R_f=0.60 (9:1 methylene chloride: methanol). Mass Spec.: M+=219 \$q=100\$.

Example 6: Preparation of 3-Amino-6(7)-Decyl-1,2,4-Benzotriazine 1,4-Dioxide

5-(1-decyl)-benzofuroxan: A combined mixture of 4-(1-decyl)-2-nitroaniline (77g, 0.28 mole), 5.25% NaOCl in H₂O (476g, 0.34 mole), 85% KOH (20.3g, 0.31 mole), Bn₄NHSO₄ (4.7g, 0.014 mole), and CH₂Cl₂ (2.28 l) was stirred rapidly for 6h and diluted with H₂O (500 ml) and CH₂Cl₂ (1 l). The separated organic phase was washed successively with 1N HCl (1 l) and brine (2 x 1 l), dried (Na₂SO₄), and concentrated in vacuo to yield a red oil, 70 g (92%).

A solution of 5-(1-decyl)-benzofuroxan as prepared above (10 g, 0.036 mole) and benzyltriethyl ammonium chloride (0.36 g, 0.0016 mole) in DMSO (180 ml) was treated gradually over several hours with cyanamide (13.0 g, 0.31 mole) and K₂CO₃ (36.8 g, 0.27 mole). The mixture was stirred for 48h and filtered. The filtrate was diluted with H₂O (6 l) and glacial acetic acid (40 ml) and extracted with CH₂Cl₂ (4 x 500 ml). The combined organic solution was washed successively with 5% NaHCO₃ solution (1 x 500 ml) and brine (2 x 500 ml), dried (Na₂SO₄), and evaporated in vacuo to dryness. The crude product was purified by chromatography on silica gel using CH₂Cl₂: methanol (98:2) to give 1.8g (16%) of compound 7 as a red

solid, m.p. 155 c (dec). Mass.

Spec.: - M+=318 (q=4), 285 (q=100).

Example 7: Preparation of 1,2,4-Benzotriazine 1.4-Dioxide

A mixture of 1.80 g (13.73 mmole) of 8, 90% H₂O₂ (9 ml) trifluoroacetic anhydride (13.5 ml) and Na₂WO₄·2H₂O (12.50g, 38 mmole) in CHCl₃ (170 ml) was stirred at room temperature for 5 days. The reaction mixture was diluted with H₂O (100 ml) and extracted with CHCl₃ (100 ml). The organic layer was washed with H₂O (50 ml), dried (Na₂SO₄), and the solvent removed in vacuo. The residue was chromatographed on silica gel using EtOAc-CH₂Cl₂ (1:1) to give 0.30 g (13.48) of compound 9 as a yellow solid, m.p. 204-205°C. Anal.

Calcd. for C₇R_sN₃O₂ (163.13): C, 51.5; H, 3.09; N, 25.76. Found: C, 51.6; H, 3.36; N, 26.01. Mass Spec.

M+=163 (q=100), 147 (q=50). TLC: R_f=0.27 (EtOAc-CH₂Cl₂, 1:1, silica gel plates). IR (nujol): 1600, 1460, 1300, 1230. UVmax (H₂O): 227 (# 22,900) 252 (# 12,950); 392 (e 4,080).

Example 8: Preparation of 7-Chloro-3-Hydroxy-1,2,4-Benzotriazine 1.4-Dioxide

A mixture of 1.50 g (7.63 mmole) of 10 in 100 ml acetic acid was treated with 2.52 g (7.63 mmole) of Na₂WO₄·2H₂O and 30 ml of 30% H₂O₂. The mixture was stirred and heated for 6 days at 50°C, then slowly evaporated to dryness to remove H₂O₂. The residue was boiled in 250 ml H₂O and filtered to remove about 25 mg of starting material 12. The aqueous solutions were then extracted with 2 x 250 ml portions of ethyl acetate. A deep red crystalline material that was characterized as 12 by TLC and Mass Spec. analysis formed in the partitioning mixture above and was collected by filtration to afford 60.0 mg of a yellowish orange solid (3.7% yield), characterized as follows as 12, which showed good solubility in a mixture of hot isopropyl alcohol and water. Mass. Spec.: M+=212 (q=100)(compound 10); TLC: R_f= 0.34 (acetone, silica gel plates).

The ethyl acetate solutions above, separated from the H₂O layer after the filtration to remove 12, were evaporated to dryness. The residue was then treated with isopropyl alcohol at room temperature to afford a dull orange solid, 0.41g (25% yield) of 11.

Mass. Spec.: M+=213 (q=70); TLC: R_f=0.22 (acetone, silica gel plates). Compound 11 was characterized as the ammonium salt, C₇H₄ClN₃O₃·NH₃, m.w. 230.61, as follows. The free acid 11 was dissolved in concentrated NH₄OH and then chilled in ice and filtered to remove a trace of insoluble 12. The red filtrate and washings were evaporated to dryness, leaving a reddish-orange solid. The solid was treated with 50 ml of boiling 1,2-dimethoxyethane, collected on a filter and washed with an additional 25 ml of hot 1,2-dimethyl ether. The solid was dried over P₂O₅ at 56°C/1.0 mm, leaving 0.244 g (87% yield) of 13.

Anal. Calcd. for C₇H₄ClN₃O₃·NH₃ (230.61): C, 36.5; H, 3.06; N, 24.30. Found: C, 36.5; H, 3.07; N, 23.94.

UVmax (H₂O): 219 (# 12,580); 265.4 (# 40,000); 4830486 (# 6,640).

Example 9: In Vivo Assay for Activity in Combination with Radiation

The compounds of the invention were tested in vivo for activity by the assay of Brown, J.M., Radiation Res (1975) 64:633-47, incorporated herein by reference.

For this assay, SCCVII carcinomas in female J mice weighing 20-25 g were used. These mice were bred under specific pathogen-free conditions and were 3-4 months old at the beginning of each experiment. The SCVIII tumor was grown intradermally in the flank from an inoculation of 2 x 10⁵ tumor cells taken from the 2nd-8th in vitro passage of the tumor cells after removal from the previous in vivo tumor. Two tumors per mouse were implanted, and were used as subject tumors when they reached a volume of approximately 100 ml. At this point the tumors contained approximately 20% hypoxic cells.

The test compound was tested at a fixed injected dose of either 5 mmol/kg or 2/3 of the LD50 (whichever is lower). - Suitable controls of test compound injected but nonirradiated and saline-injected and irradiated mice were also included. A fixed radiation dose of 20 Gy was applied at variable intervals of 2 hr after to 3 hr before injection of the drug. By using these intervals, the results give an indication of both the optimum irradiation time and the extent of extra cell killing compared to radiation alone. The results of such time-course experiments using 3-amino-1,2,4-benzotriazine 1,4-dioxide are shown in Figure 2. They show enhanced cell killing compared to radiation only, more than would have been expected on the basis of additivity of the two individual cytotoxicities. The similar increased cytotoxicity when the drug is given before or after radiation indicates selective toxicity to the hypoxic cells rather than a radiosensitizing effect of the benzotriazine dioxide.

Irradiation of the SCCVII tumors was done by irradiating nonanaesthetized tumor-bearing mice in a Plexiglas box. Irradiation conditions were 250 kvp X-rays, 15 mA, FSC 33 cm, added filtration of 0.35 mm Cu, half value layer 1.3 mm Cu, and a dose rate of 317 rad/min.

The amount of cell killing was judged by survival rate of dissected and cultured tumor cells as follows. The tumor-bearing mice were killed 24 hr after irradiation, and tumors were dissected from the skin, cut into several pieces, and made into a fine brei by high-speed chopping with a razor blade attached to a jigsaw. The brei was added to 30 ml of Hank's buffered salt solution (HBSS) containing 0.028 DNase, 0.05% promase, and 0.02% collagenase. The suspension was stirred for 30 min at 37 C, filtered, and centrifuged at 1,600 rpm for 10 min at 40C. The cell pellet was resuspended in complete Waymouth's medium plus 15% fetal calf serum (FCS) and an aliquot mixed with trypan blue and counted with the use of a hemacytometer. Suitable solutions of this serum plated into 60- or 100-mm polystyrene petri dishes (Lux Scientific Corp) in 5 or 15 ml of medium. After incubation for 13 days, the colonies were fixed and stained, and those containing 50 cells or more were counted. The dilution yielding an average count of 25 100 colonies in a 60 mm dish was used in calculation of results.

Example 10: Cytotoxicity Tests

Cytotoxicity tests were carried out using 3-amino-1,2,4-benzotriazine 1,4-dioxide and a variety of aerobic and hypoxic cells in culture (human, mouse, and hamster). The cells in spinner flasks were gassed for one hour at 37 C with either air or nitrogen containing 5% CO₂ prior to adding the specified amounts of the drug. Figures 1A, 1B and 1C show the results for cell survival of mouse, hamster and human cells at various concentrations of 3-amino-1,2,4-benzotriazine 1,4-dioxide. It was found that only 1 to 2% of the drug concentration under aerobic conditions was required to get equal cell killing under hypoxia. This ratio of selective hypoxic toxicity (50-100) is higher than that for any compound so far reported in the literature.

Example 11: Determination of LD₅₀

LD₅₀ is determined in BALB/c female mice (weighing 20-25 g) following intraperitoneal (ip) injection, unless the compound tested has low lipophilicity and is very soluble, wherein intravenous (iv) administration is used. LD₅₀ values at 1, 2, 5, and 60 days are determined by administering graded doses of the drug dissolved in physiological saline immediately prior to injection.

Example 12: Radiosensitivity in Vitro

The results of assays to determine the concentration of drug necessary to produce a sensitizer enhancement ratio of hypoxic cells in culture of 1.6 are as follows: Compound Cl.6 (mM)

7-chloro-3-amino-1,2,4-benzotriazine 3.3

1-oxide 6(7)-methoxy-3-amino-1,2,4-benzotriazine 1,4-dioxide 1.0

3-hydroxy-1,2,4-benzotriazine 1,4-dioxide 2.0

Modifications of the above described modes for carrying out the invention that are apparent to those of skill in the chemical, pharmaceutical, medical, and related arts are intended to be within the scope of the following claims.

Example 13: Enhanced Tumor Cell Tonicity Using Hydralazine

Hydralazine is an antihypertensive drug which acts by relaxing the smooth muscle around blood vessels.

This has the effect of preferentially shunting blood flow into normal tissues and away from tumors, which

process produces immediate hypoxia in the tumors. If 3-amino-1,2,4-benzotriazine 1,4-dioxide is given in conjunction with this agent, there is a massive increase in tumor cell killing. In this experiment, neither hydralazine nor the aforementioned benzotriazine compound produced any significant cell killing in the SCCVII tumor, whereas the combination of the two reduced survival by a factor of 103 (i.e., only 1 cell in every 1000 was left viable). The experimental procedures are the same as described in Example 9, and the results are shown in Figure 3.

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Claims

Claims

1. Method of selectively killing hypoxic tumor cells comprising administering directly to said cells a compound of the formula

wherein X is H, hydrocarbyl (1-4C), NH₂, NHR or NR₂ where each R is independently an alkyl of 1-4 carbon atoms, an amide, or a morpholino moiety and may further be substituted with hydroxy, alkoxy, amino, or halogeno substituents;

n is 1; and

y₁ and y₂ are independently either H, halogeno, hydrocarbyl (1-14C) including cyclic and unsaturated hydrocarbyl, optionally substituted with 1 or 2 substituents selected from the group consisting of halogeno, hydroxy, epoxy, alkoxy, alkylthio, amino (including morpholino), acyloxy, acylamido and their thio analogs, carboxy, alkoxy carbonyl, carbamyl or alkyl carbamyl, and wherein the hydrocarbyl can optionally be interrupted by a single ether (-O-) linkage, or wherein y₁ and y₂ are independently either NHR', O(CO)R', NH(CO)R', O(SO)R', or O(POR')R' in which R' is a hydrocarbyl optionally substituted as defined above.

2. Method of radiosensitizing hypoxic tumor cells, comprising administering a compound of the formula:

wherein X is H, hydrocarbyl (1-4C), OH, OR, NH₂, NHR or NR₂ where each R is independently an alkyl of 1-4 carbon atoms or a morpholino moiety and may further be substituted with hydroxy, alkoxy, amino, or halogeno substituents;

wherein n is 0 or 1; and

y₁ and y₂ are independently either halogeno, hydrocarbyl (1-14C) including cyclic and unsaturated hydrocarbyl, optionally substituted with 1 or 2 substituents selected from the group consisting of halogeno, hydroxy, epoxy, alkoxy, alkylthio, amino (including morpholino), acyloxy, acylamido and their thio analogs, carboxy, alkoxy carbonyl, carbamyl or alkyl carbamyl, and wherein the hydrocarbyl can optionally be interrupted by a single ether (-O-) linkage, or wherein y₁ and y₂ are independently either NHR', O(Cp)R', NH(CO)R', O(SO)R', or O(POR')R' in which R' is a hydrocarbyl optionally substituted as defined above.

3. Compounds given by the following formula:

wherein when X is H, hydrocarbyl (1-4C), OH,

OR, or NR₂ and n is 0 or 1, or when X is NH₂ or NHR and n is 0, where each R is independently an alkyl of 1-4 carbon atoms or a morpholino moiety and may further be substituted with hydroxy, alkoxy, amino or halogeno substituents, y₁ and y₂ are independently either H, halogeno, hydrocarbyl (1-14C) including cyclic and unsaturated hydrocarbyl, optionally substituted with 1 or 2 substituents selected from the group consisting of halogeno, hydroxy, epoxy, alkoxy, alkylthio, amino (including morpholino), acyloxy, acylamido and their thio analogs, carboxy, alkoxy carbonyl, carbamyl or alkyl carbamyl, and wherein the hydrocarbyl can optionally be interrupted by a single ether (-O-) linkage, or wherein y₁ and y₂ are independently either NHR', O(CO)R', NH(CO)R', O(SO)R', or O(POR')R' in which

R' is a hydrocarbyl optionally substituted as defined above, with the proviso that when y₁ and y₂ are hydrogen, X is other than methyl; and

wherein when X is NH₂ and n is 1, y₁ and y₂ are independently either H, saturated or unsaturated hydrocarbyl of between about 7 and 14 carbon atoms, unsaturated hydrocarbyl of between about 1 and 6 carbon atoms, either hydrocarbyl substituent being unsubstituted or substituted with halogen, hydroxy, epoxy, alkoxy, alkylthio, amino, morpholino, acyloxy, acylamido and their thio analogs, and where the hydrocarbyl can be optionally interrupted by a single ether linkage, or wherein y₁ and y₂ are independently either NHR', O(CO)R', NH(CO)R', O(SO)R' or O(POR')R', in which R' is a hydrocarbyl optionally substituted as defined above.

4. A method of making a compound of the formula

by reacting the corresponding 3-amino l-oxide derivative with hydrogen peroxide in the presence of $\text{Na}_2\text{WO}_4 \cdot 2\text{H}_2\text{O}$ at a temperature of at least about 50 C.

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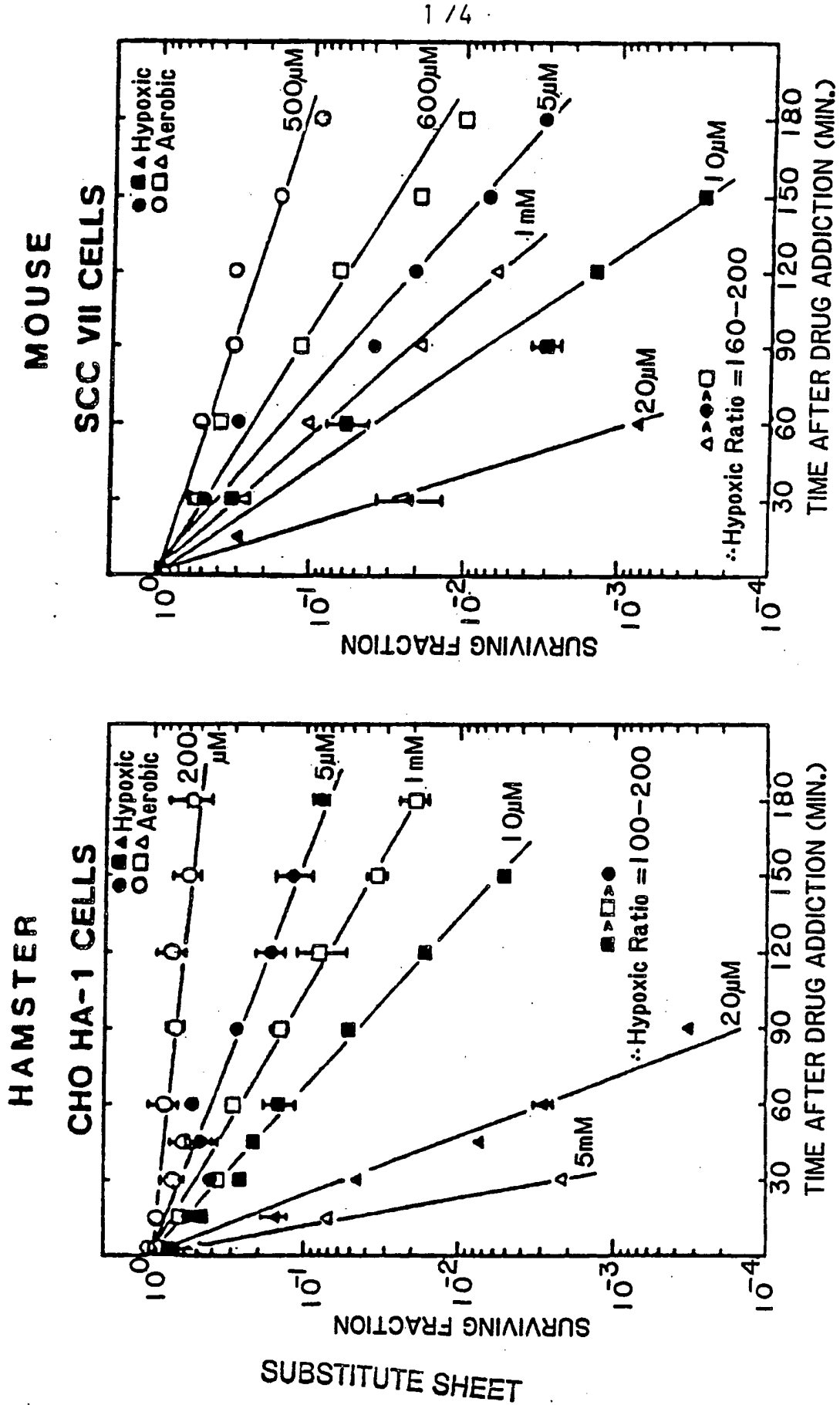
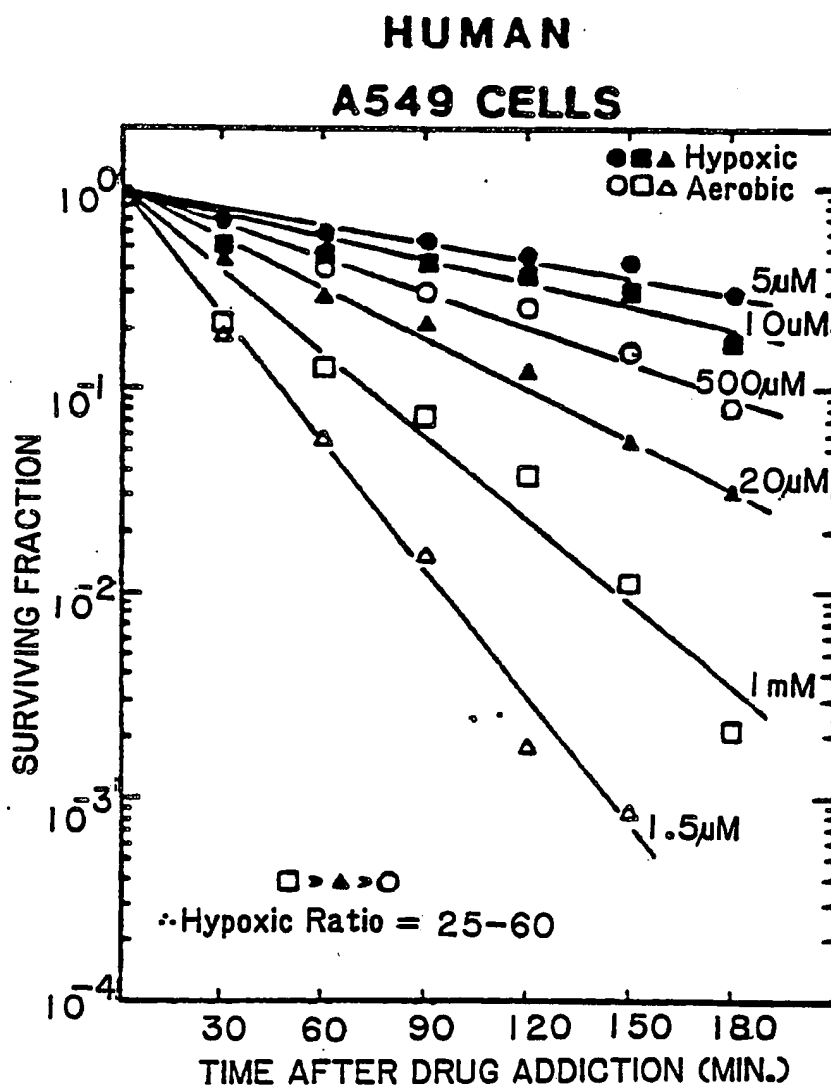


FIG. 1B

**FIG. 1C****SUBSTITUTE SHEET**

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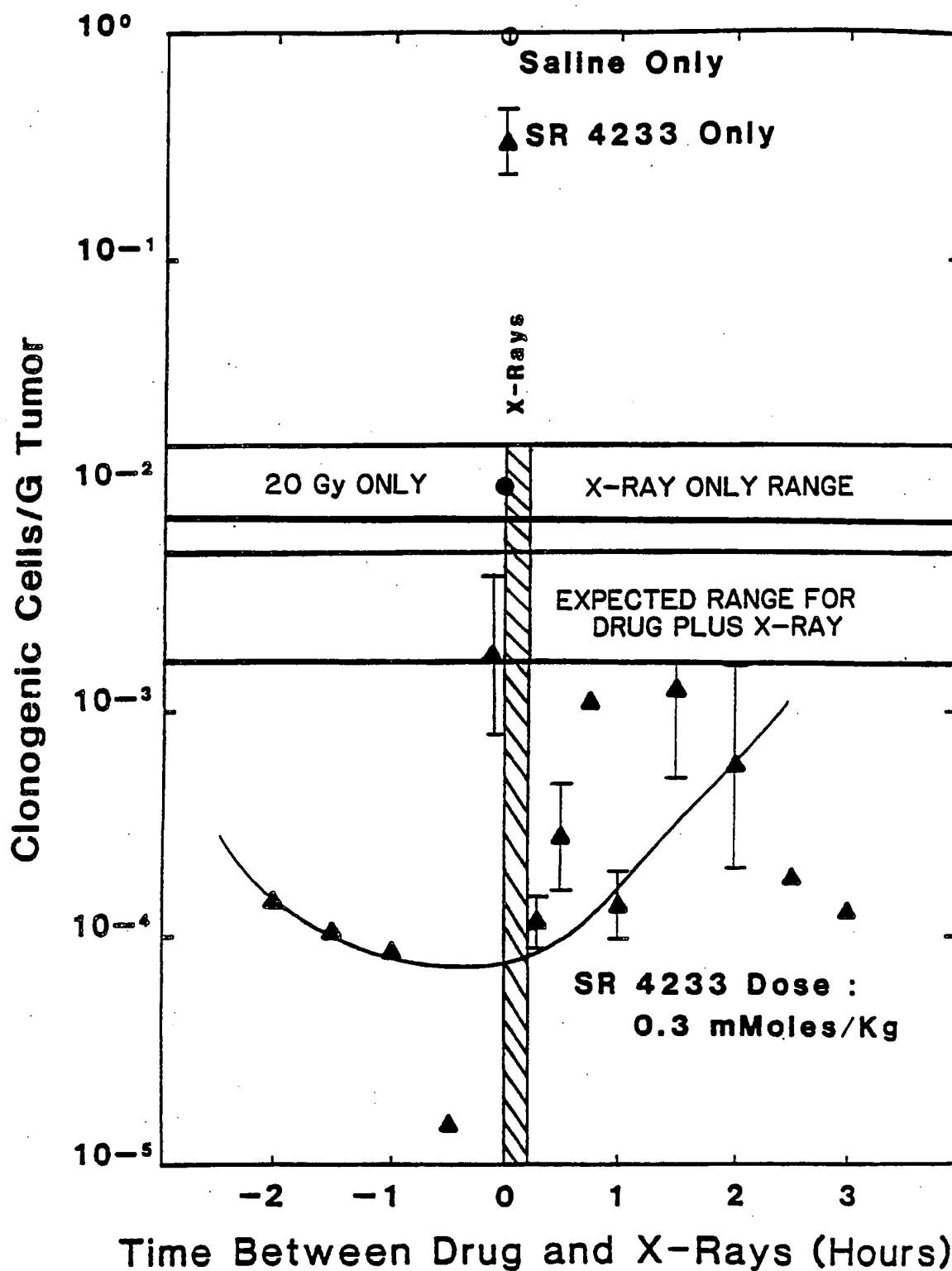


FIG. 2

SUBSTITUTE SHEET

CYTOTOXICITY OF HYDRALAZINE
AND SR 4233 IN SCCVII TUMORS

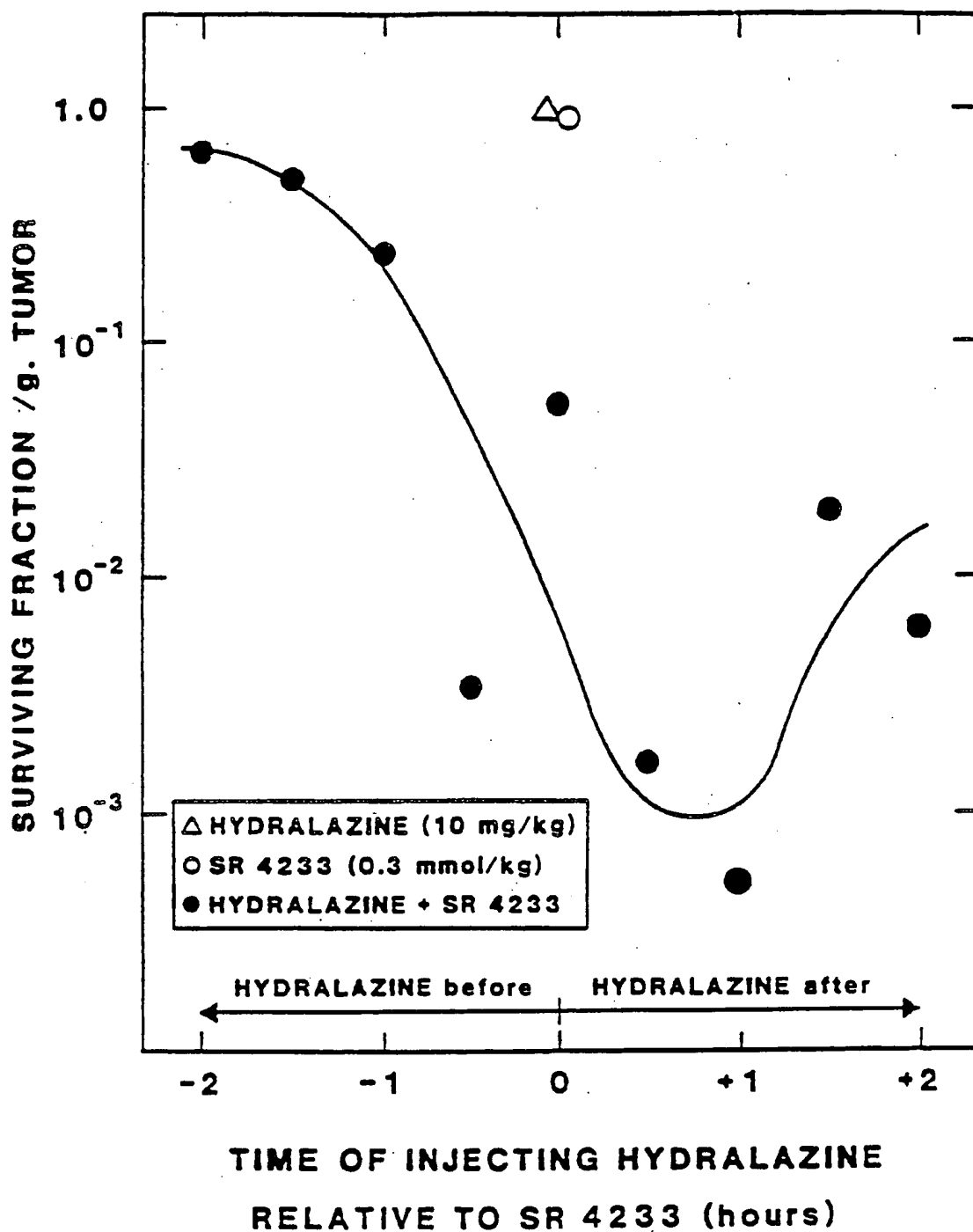


FIG. 3

SUBSTITUTE SHEET